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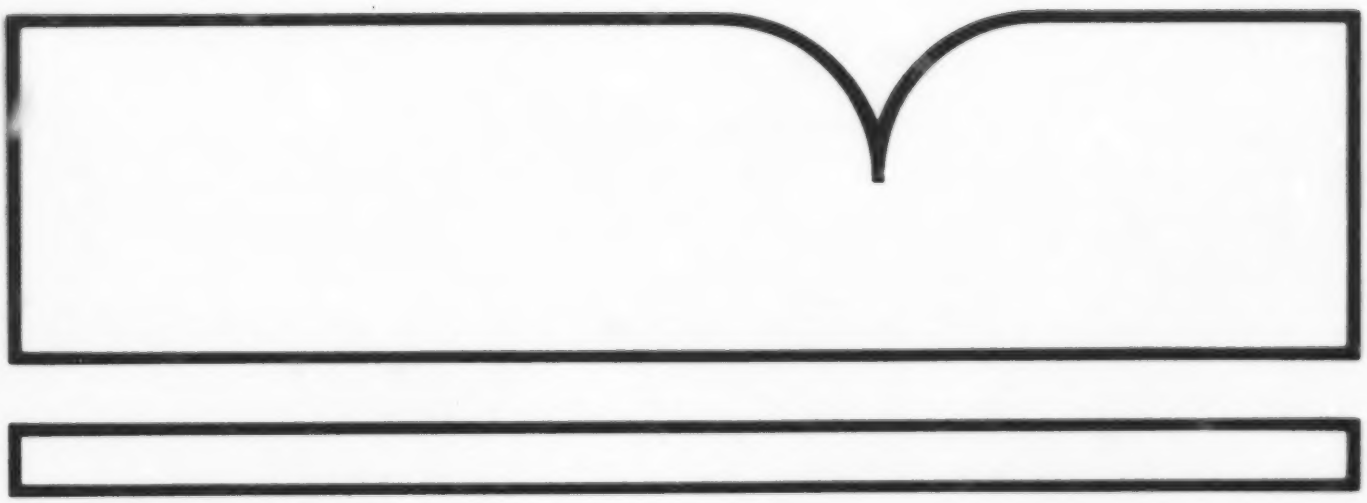

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VIRAL GASTROENTERITIS AGENTS AND WATERBORNE DISEASE

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16. ABSTRACT

The application of electron microscopic techniques in the study of human gastroenteritis led in the 1970's to the identification of new viral agents that had previously escaped detection by routine cell culture procedures. These agents have been the focus of study by researchers in many areas of the world and improved detection methods for these agents continue to be developed. Two or the best-known agents, rotavirus and Norwalk virus, have been implicated in waterborne outbreaks of this illness. Another virus, the Snow Mountain agent, was first identified from the investigation of one outbreak.

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Abstract

The application of electron microscopic techniques in the study of human gastroenteritis led in the 1970's to the identification of new viral agents that had previously escaped detection by routine cell culture procedures. These agents have been the focus of study by researchers in many areas of the world and improved detection methods for these agents continue to be developed. Two of the best-known agents, rotavirus and Norwalk virus, have been implicated in waterborne outbreaks of this illness. Another virus, the Snow Mountain agent, was first identified from the investigation of one waterborne outbreak.

Key Words: Norwalk virus, Rotavirus, Snow Mountain agent, viral gastroenteritis agents, waterborne outbreaks.

It has been almost fifteen years since Norwalk virus was first visualized by investigators in the US. That successful application of electron microscopic techniques in the study of infectious gastroenteritis was soon followed by reports of other virus-like particles being observed by investigators in other countries. Cell culture, the traditional procedure used by virologists for virus isolation, had failed to detect these newly-observed agents. It soon became clear that the detection of these non-cultivable viruses by electron microscopy (EM) was opening new avenues for research on infectious gastroenteritis. By 1976, six morphologically distinct viral agents had been visualized and had been implicated, to varying degree, in the etiology of the illness. These agents included: the Norwalk virus, rotavirus, adenovirus, a coronavirus-like agent, astrovirus, and calicivirus. They were originally reported by investigators in the US (Kapikian, et. al., 1972), Australia (Bishop, et. al., 1973), the UK (Caul, et. al., 1975; Flewett, et. al., 1973, 1974; Madeley and Cosgrove, 1975, 1976), and India (Mathan, et. al., 1975) (Table 1).

The excellent resolution of virus detail that was obtained with negatively-stained fecal preparations provided striking images of virus particles that could be identified by appearance, alone. As these viruses were frequently shed in large numbers by infected individuals, they could often be observed in stools without extensive concentration or purification. With certain viruses, the technique of immune electron microscopy (IEM) was used for virus detection. This technique incorporated the use of antibody-containing, convalescent-phase serum to aggregate and coat the virus particles. Such antibody-aggregated and coated particles proved to be highly visible after negative-staining. The technique was particularly useful when virus was present in low concentration; however, antibody-coated particles tended to have obscured structural detail. Seroconversions, resulting from the production of virus-specific antibody in

infected individuals, were also detected by IEM. The visualization of the Norwalk virus using IEM clearly demonstrated the effectiveness of the technique.

The Viral Agents of Gastroenteritis. (Figure 1 and 2)

Norwalk virus was identified from the investigation of a 1968 outbreak that occurred in Norwalk, Ohio. The virus particle is 27-32 nm in size and possesses characteristic but poorly-defined surface structure. The nucleic acid content is not known but protein analysis has revealed a single structural protein with a molecular weight of 59,000 (Greenberg, et. al., 1981). The virus is responsible for outbreaks of gastroenteritis among people of all ages, except for the very young. The virus is the prototype for other "Norwalk-like" or "small round structured" viruses (Table 2). These viruses also exhibit a poorly-defined surface structure, but they can be distinguished from the featureless virus particles which make up the parvovirus and picornavirus groups (Caul and Appleton, 1982). These distinctive agents were identified during outbreak investigations (Caul, et. al., 1979; Dolin, et. al., 1982; Kogasaka, et. al., 1981; Oshiro, et. al., 1981; Taniguchi, et. al., 1979; Wyatt, et. al., 1974) or illness studies (Kogasaka, et. al., 1980; Middleton, et. al., 1977; Oishi et. al., 1985; Spratt, et. al., 1978), and some have since been shown to be antigenically related. Such is the case with the Otofuke, SRV'76, and Sapporo agents. Limited availability of characterized material and differences in geographic location have restricted comparative study on most of the agents. However, in the comparative studies that have been done, it has been found that Hawaii, the Otofuke group, Marin, and Snow Mountain agents are antigenically distinct from the Norwalk virus. Failure to propagate these agents in cell culture has severely hampered further characterization.

Rotavirus, on the other hand, has proven more adaptable to cell culture procedures and has been intensely studied in recent years. Characteristic rotavirus particles are about 70 nm in diameter and, when suitably penetrated by negative stain, have a wheel-like appearance. The virus has been classified as a separate

genus in the Reoviridae family. It has a segmented genome of double-stranded RNA, and there are at least four distinct serotypes of human rotavirus identified by tube neutralization or plaque reduction tests (Wyatt, et. al., 1983). The original rotaviruses isolated from man and other species shared a common group antigen; however, rotavirus particles were soon found that lacked this common antigen. These particles were termed "atypical rotaviruses", "pararotaviruses", or "rotavirus-like agents". They were observed in several species, including man, and have since been grouped according to antigenic and genomic similarity into group B and group C rotaviruses (Pedley, et. al., 1983). The original common-antigen sharing viruses constitute the group A rotaviruses. Rotavirus has emerged as one of the major etiologic agents of gastroenteritis in infants and young children and has worldwide impact. Various studies indicate 20-60% of the diarrheal cases requiring hospitalization to be associated with rotavirus (Flores, et. al., 1986). Because rotaviral gastroenteritis is often associated with severe dehydration, the virus undoubtedly contributes substantially to the high infant mortality rates of developing countries. Rotaviral gastroenteritis can afflict adults, although in adults the illness is generally mild.

The 30-40 nm calicivirus particles are identified by the darkly-staining cup-like depressions observed on their surface. Prior to their detection in man, caliciviruses were known to be pathogenic agents in pigs, cats, and sea lions. The caliciviruses possess a single structural protein with molecular weights ranging between 60,000 and 70,000 (Schaffer, et. al., 1980). This structural similarity to the Norwalk virus, and more recently to the Snow Mountain agent, suggests these viruses may be related. Gastroenteritis outbreaks among infants and school children have been reported in both the UK and Japan (Cubitt, et. al., 1979, 1980; Oishi, et. al., 1980). In the UK, outbreaks involving the elderly have been reported (Cubitt, et. al., 1981; Humphrey, et. al., 1984). IEM evidence suggests that different human serotypes may exist. Recent success in cell-culture propagation of a human

calicivirus has been reported (Cubitt, et. al., 1984). The human virus, as with the caliciviruses of other species, has an RNA genome.

Astrovirus particles are 28-30 nm in diameter. When negatively stained, a star-like shape is visible on suitably oriented particles. Five human serotypes have been differentiated using an innumofluorescent test and immunosorbent electron microscopy (Kurtz and Lee, 1984). The virus is found in a number of species. Research on the virus found in sheep demonstrated the genome to consist of single-stranded RNA. A successful propagation of the human virus has been reported (Lee and Kurtz, 1981). Gastroenteritis outbreaks among infants, children, and some adults have been reported in Europe and Asia (Konno, et. al., 1982; Kurtz, et. al., 1977). The virus appears to be associated with a rather mild form of the illness.

The general morphology of adenovirus has been recognized for over 25 years (Horne, et. al., 1959). The large, icosahedral particles are 70-90 nm in size, and a highly visible pattern of capsomers is exhibited on the surface of these particles. Although adenovirus was considered a virus of the respiratory tract, it had frequently been isolated from feces in cell culture. It was no surprise when adenovirus particles were visualized in stools by EM (Flewett, et. al., 1974). Interestingly, however, the visualized adenovirus particles could not generally be isolated in cell culture (Gary, et. al., 1979). These non-cultivable viruses came to be known as "enteric" or "fastidious" adenoviruses. Eventually these enteric adenoviruses, which include serotypes 40 and 41, were cultivated using non-routine cell cultures. In the developed countries, these viruses may be second only to rotavirus as agents of infantile gastroenteritis. However, much concerning their significance remains to be determined (Albert, 1986; Madeley, 1986).

Pleomorphic particles resembling coronaviruses have also been frequently observed in stools (Marshall, et. al., 1982; Payne, et. al., 1986). They range in size from 60 nm to 1200 nm and are distinguished by a 20-40 nm fringe or "corona" of knob-like structures

that surround the particles. This fringe is slightly different from the typical coronavirus fringe which consists of bulbous or petal-shaped projections. Although typical coronaviruses do cause gastroenteritis in animals, it is far from certain that these coronavirus-like particles have a role in the human illness (Macnaughton and Davies, 1981).

Waterborne Outbreaks of Viral Gastroenteritis.

Despite the difficulty of conclusively determining both a waterborne mode of transmission and a viral cause, there is an increasing amount of evidence that implicates at least three viral agents in waterborne outbreaks of gastroenteritis. The three agents that have thus far been implicated are: Norwalk virus, rotavirus, and the Snow Mountain agent.

A review of nonbacterial gastroenteritis outbreaks by the Centers for Disease Control, covering 1976 through 1980, attributed 31 of 74 outbreaks to the Norwalk virus (Kaplan, et. al., 1982). In those 31 and 7 additional outbreaks confirmed at the National Institutes of Health, water was identified as the vehicle of transmission in 13 outbreaks. Municipal water systems were implicated in 2 of the 13 outbreaks, and 7 were associated with semi-public water supplies. The remaining 4 outbreaks were swimming-related or involved cruise-ship water supplies. Since 1980, CDC has reported 3 waterborne outbreaks, occurring in 1982, and one 1984 outbreak to have been caused by Norwalk virus (Centers for Disease Control, 1985).

Four outbreaks involving drinking water supplies have been individually described in the literature (Table 3). In these outbreaks, Norwalk infection of afflicted individuals was identified by seroconversion to the agent, as demonstrated by radioimmunoassay. Data gathered from questionnaires and telephone surveys were used to associated the illness with the consumption of drinking water or being served by a particular water system.

The first of these outbreaks occurred in May of 1978 at an elementary school in Pierce Co., Washington (Taylor, et. al.,

1981). Approximately 430 students, teachers, and support personnel were directly affected. Back-syphonage of sewage into the school's water supply appeared to be responsible for the outbreak.

In July of 1978, an outbreak occurred at a children's summer camp in Monroe Co., Pennsylvania (Wilson, et. al., 1982). One hundred and ninety-three cases of illness were reported and involved two consecutive camp sessions. Although no cross-contamination of the camp's water system was identified, fecal coliforms were found in the camp's unchlorinated well water.

Approximately 1500 people may have been afflicted in Rome, Georgia during an outbreak in August of 1980 (Kaplan, et. al., 1982). The outbreak was the first to involve a large municipal water system. Epidemiologic evidence indicated the outbreak was caused by local contamination of the water system by an adjacent textile plant system that was used for industrial purposes.

In January of 1982, an outbreak occurred in the small rural community of Tate, Georgia (Goodman, et. al., 1982). Approximately 500 of the 800 people served by the community water system were afflicted. Fecal coliforms were identified in a tap water specimen that had undetectable residual free chlorine. Two possible sites for contamination of the water system were identified.

Although the Norwalk virus has been associated with most waterborne outbreaks in which a gastroenteritis virus has been identified, there have been a number of outbreaks in which rotavirus has been implicated. Seven rotavirus-associated outbreaks have been reported in the literature, and these have occurred in seven different countries (Table 4).

The first rotavirus-associated outbreak occurred in a small town in Sweden, in January of 1977 (Lycke, et. al., 1978). Over 3000 cases of gastroenteritis were registered and involved approximately 30% of the town's population. EM examination of stool samples identified rotavirus in 17 of 36 specimens. An additional 30 nm particle was observed in three rotavirus-containing stools and may have had some involvement in the outbreak. The report indicated that the town's fresh water supply had been contaminated

with sewage effluent.

The next outbreak occurred in May of 1980 at a private school in Rio de Janeiro, Brazil (Sutmoller, et. al., 1982). Approximately 800 of 1150 students were affected. Rotavirus was detected by IEM and enzyme immunoassay in 11 of 19 stool samples. Shigella sonnei was identified in 4 of these 11 stools and in 6 others, indicating at least two pathogens were involved in the outbreak. Accidental use of a shallow well that was known to contain high coliform counts appeared to be responsible for the outbreak.

In March of 1981, two small Colorado communities that were served by the same water district experienced an outbreak of the illness (Hopkins, et. al., 1984). Epidemiologic analysis indicated approximately 1500 people were ill during the outbreak, and the illness was statistically associated with water consumption. Stool and paired serum examination by direct EM, IEM, and enzyme immunoassay identified rotavirus infection in 5 of 7 ill individuals. Tests for other enteric pathogens were negative. A chlorinator failure and sludge contamination of source water were responsible for the outbreak.

An outbreak that occurred in March and April was described in a 1981 report from the Soviet Union (Zamotin, et. al., 1981). The outbreak occurred at a settlement and involved at least 173 illness cases. Investigation of the outbreak revealed contamination of the water system as the likely cause. Virological examination revealed rotavirus in 10 of 24 stools from ill individuals.

In November of 1981, an outbreak began at a large East German town (Walter, et. al., 1982). Between November and January, almost 12,000 illness cases were reported and involved almost 4% of the town's population. Rotavirus was visualized by EM in 7 of 14 randomly selected stool specimens. The town is situated along a river, and the outbreak coincided with the flooding of the town's wells, at a time when chlorination practice was being changed.

More than 12,000 adults were afflicted in two separate outbreaks of gastroenteritis that occurred in China, in late 1982 and early 1983 (Tao, et. al., 1984). The outbreaks occurred near Lanznou and near Jinzhou and were the first to be associated with

atypical rotavirus. Rotavirus particles were found in 101 of 106 fecal concentrates by EM. The virus has recently been identified as belonging to the group B rotaviruses (Chen, et. al., 1985) Data gathered on the Jinzhou outbreak indicated that the illness was initially transmitted through a contaminated water supply.

Most recently, a 1984 summary of waterborne outbreaks that occurred in Israel during the period, 1976-1980, reported one outbreak in which rotavirus was identified (Tulchinsky, et. al., 1984). The outbreak involved a community water supply that was found to have been contaminated. An unspecified number of people were afflicted during the outbreak. Rotavirus was detected in the stools of 3 of 17 ill individuals, from whom no bacterial enteropathogens were identified.

Both rotavirus and Norwalk virus had been recognized as viral agents causing gastroenteritis before their implication in waterborne outbreaks. However, the Snow Mountain agent was first identified as a result of the investigation of one 1976 waterborne outbreak (Morens, et. al., 1979). The outbreak occurred in December at a resort camp in Granby, Colorado and afflicted at least 418 of 1200 people visiting the camp. Epidemiologic investigation demonstrated an increased attack rate with increased water consumption, and a leaking septic-tank system was determined to have contaminated the camp's water supply. The Snow Mountain agent was visualized by IEM in the stools of 2 of 5 ill individuals. A seroconversion to the agent was demonstrated in 3 individuals. Comparison studies have revealed the agent to be antigenically distinct from Norwalk, Hawaii, and Marin viruses, although these viruses appear similar morphologically (Dolin, et. al., 1982). Rotavirus involvement in this outbreak was ruled out.

Needs and Developments in Virus Detection.

In the waterborne outbreaks that have been described, identification of the responsible viruses has depended on the examination of stools and paired sera from afflicted individuals. EM methods are generally not sensitive enough for the direct examina-

tion of drinking water for virus. Although greatly dependent on the skill and experience of the microscopist, a virus concentration of 10^5- 10^6 particles per ml is required for visualization. Immunoassays, such as ELISA and RIA, have proven more practical than EM for the assay of large numbers of clinical specimens, but these too have been poorly-suited for the detection of low numbers of virus in water supplies. However, developments in cell culture, the use of nucleic acid probes, and continued improvements in immunoassays are expected to greatly reduce the amount of virus needed for detection. When coupled with efficient concentration procedures, effective methods for the detection of gastroenteritis viruses in water should become a reality. Enterovirus concentration procedures have already been tested and modified using cultivable rotaviruses (Guttman-Bass and Armon, 1983; Ramia and Sattar, 1980; Smith and Gerba, 1982). In these procedures, the virus, suspended in water, isadsorbed under optimized conditions to a filter element or to a layer of talc-Celite. The virus is then eluted from the adsorbing material with a beef extract suspension or with tryptose phosphate broth. As the eluted volume is generally too large to be practically assayed, the eluted virus can be further concentrated by organic flocculation or hydroextraction before final assay. Virus recoveries from these concentration procedures vary greatly, particularly when large water volumes are processed, but these procedures have been successfully used in the detection of rotavirus in drinking and environmental water (Deetz, et. al., 1984; Keswick, et. al., 1984; Raphael, et. al., 1985).

Quantifiable assays that measure viable virus particles are a prerequisite for obtaining data on the susceptability of gastroenteritis viruses to disinfection procedures. Such assays do exist for rotavirus, and disinfection studies have been done using both simian and a cultivable human rotavirus (Berman and Hoff, 1984; Vaughn, et. al., 1986). Results of these disinfection studies revealed both rotaviruses to be rapidly inactivated by free chlorine levels as low as 0.3 mg/liter. The current data using these laboratory strains suggests rotavirus presents no unusual problems for the chlorine disinfection practices of water suppliers. However,

naturally-occurring rotavirus and enterovirus have been detected in the finished water of a treatment plant located in a developing country (Keswick, et. al., 1984). In-depth study of that plant has suggested that conditions can exist that favor the survival of virus in a generally sound treatment process. Inadequate floc formation, floc breakdown, and filter overloading are conditions that can lead to increased amounts of particulates in treated water and decrease the effectiveness of terminal disinfection. Such observations emphasize the importance of having effective methods for assaying water supplies for pathogenic viruses.

Limited disinfection data is available for other gastroenteritis viruses. One inactivation study with the Norwalk virus, however, suggests that Norwalk virus is very resistant to chlorine inactivation (Keswick, et. al., 1985). Such resistance may account for its frequent involvement in waterborne outbreaks.

Significance of Waterborne Viral Gastroenteritis.

Waterborne transmission of gastroenteritis viruses is one component of a truly massive problem that confronts the world today. In Africa, Asia, and Latin America, 1000 million episodes of acute diarrheal disease occur annually in children under 5 years of age. The disease has been estimated to account for 4.6 million deaths per year among these children (Snyder and Merson, 1982). Such statistics reflect the poor conditions under which two-thirds of the world's population live. Inadequate and contaminated water supplies, and poor sanitation are fundamental causes for the widespread transmission of this disease throughout developing nations. Substantial efforts are being made in many countries to address these unfortunate conditions. However, with explosive population growth compounding the problem, substantial improvement for many, may not soon be realized. Fortunately, widespread implementation of programs of oral rehydration therapy is expected to have a major impact on reducing deaths among the severely afflicted. With such programs in place, the World Health Organization projects that by the end of the decade 1.5 million childhood deaths due to

diarrhea will be prevented annually (Mason, 1986). As rotavirus is the most common etiologic agent among the severely afflicted, development of an effective vaccine against the virus would also have a significant impact on reducing deaths. Clinical trials of candidate vaccines are currently under way.

In the developed nations, acute gastroenteritis does not [illegible]rally result in death. However, it is a very common illness [illegible]sults in substantial loss of time at work and school. In t[illegible], [illegible]ral gastroenteritis outbreaks that are waterborne in nature are a sporadic occurrence. Such outbreaks are voluntarily reported by state health departments to CDC, where they are tabulated and reported in an annual summary of waterborne disease outbreaks. It is acknowledged that the reported outbreaks represent only a fraction of those actually occurring. When requested, CDC and EPA, cooperatively, have assisted state and local authorities in outbreak investigations.

In the most recent annual summary, covering the year 1984, 14 states reported 26 waterborne disease outbreaks that involved a total of 1800 illness cases (Centers for Disease Control, 1985). The previous year, 19 states reported 40 outbreaks that involved 21,000 illness cases (Centers for Disease Control, 1984). In these two years, only one gastroenteritis outbreak was attributed to a recognized viral agent (Norwalk virus). However, there were 24 gastroenteritis outbreaks of unknown etiology that involved 17,000 illness cases. As it is likely that many of these outbreaks were caused by gastroenteritis viruses that went undetected, it is evident that more effective tools for detecting these agents are needed.

Water system deficiencies that have caused or contributed to most waterborne disease outbreaks in the US are the use of untreated, contaminated water supplies and poor practices in water treatment and distribution (Lippy and Waltrip, 1984).

Although it is difficult to objectively assess the physical discomfort of individuals afflicted in waterborne outbreaks, such outbreaks do have measurable economic impact. In the 1981 rotavirus

associated outbreak in Colorado, direct costs to the involved communities were estimated to amount to 73 dollars per resident (Hopkins, et. al., 1986). Such costs included: medical care, time lost from work, bottled water, and emergency repairs to the water treatment system. However, whether judged from an economic or a public health perspective, it is clear that communities can ill afford to neglect their water supply operations.

Today, despite 15 years of investigation, there is much that remains to be determined about the agents that cause human viral gastroenteritis. Currently available data on their association with waterborne disease is particularly limited. As methods for detecting these agents in water develop and become more widely available, a clearer understanding of their significance as waterborne disease pathogens will emerge.

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MICROSCOPICALLY DISTINCT AGENTS IDENTIFIED
IN HUMAN ILLNESS STOOLS

Agent	Year*	Location
Norwalk virus	1972	US
Rotavirus	1973	Australia, UK
Adenovirus	1974	UK
Coronavirus(-like)	1975	India, UK
Astrovirus	1975	UK
Calicivirus	1976	UK

*date of report

Table 1

27-40 NM AGENTS WITH CHARACTERISTIC BUT POORLY-DEFINED STRUCTURE
(NORWALK-LIKE OR SMALL ROUND STRUCTURED VIRUSES)

Agent	Year*	Location
Norwalk virus - prototype	1972	US
Hawaii virus	1977	US
Minireo/Minirotavirus	1977/78	Canada
Otofuke/SRV'76/Sapporo agents	1979/80/81	Japan
Taunton agent	1979	UK
Osaka agent	1985	Japan
Possible candidates:		
Marin agent	1981	US
Snow Mountain agent	1982	US

*date of report

Table 2

FOUR WATERBORNE OUTBREAKS - NORWALK VIRUS

Location, Year	Ill	Remarks
Washington, 1978	430*	Elementary school
Pennsylvania, 1978	193	Summer camp
Georgia, 1980	1500	System near textile plant
Georgia, 1980	500	Deficient rural system

*calculated from reported attack rate

(adapted from Williams and Akin, 1986)

Table 3

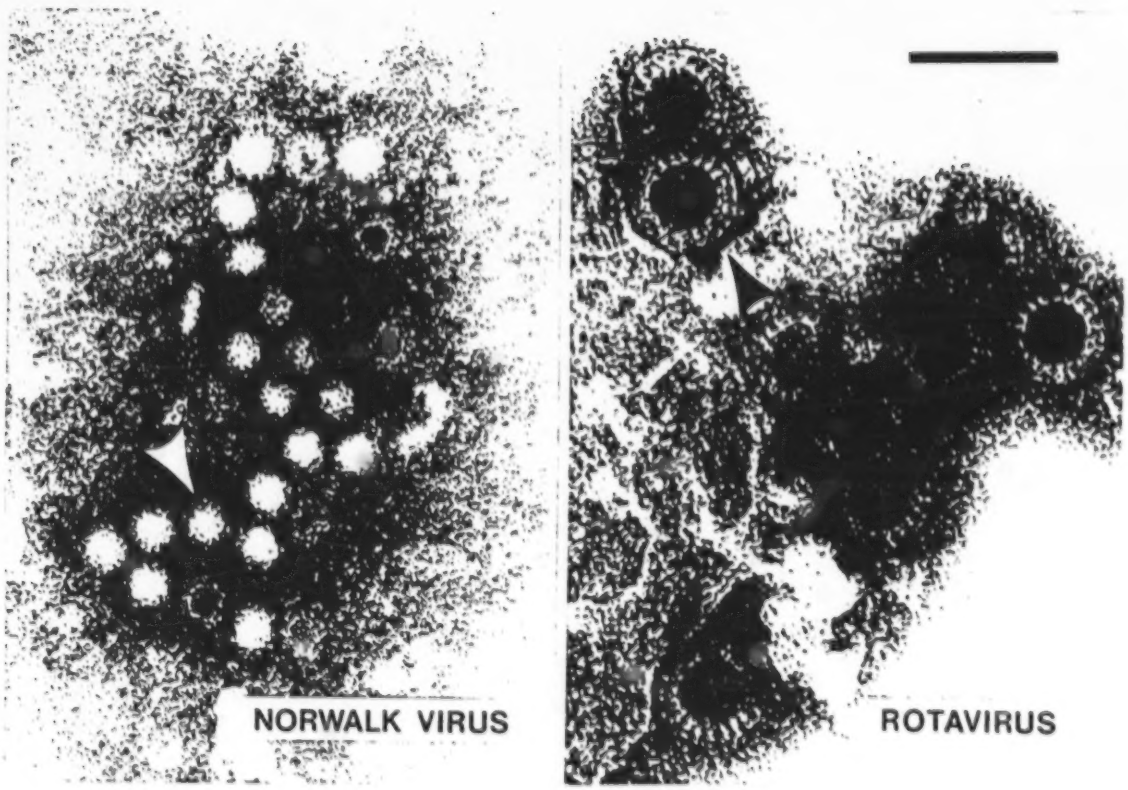
WATERBORNE OUTBREAKS - ROTAVIRUS

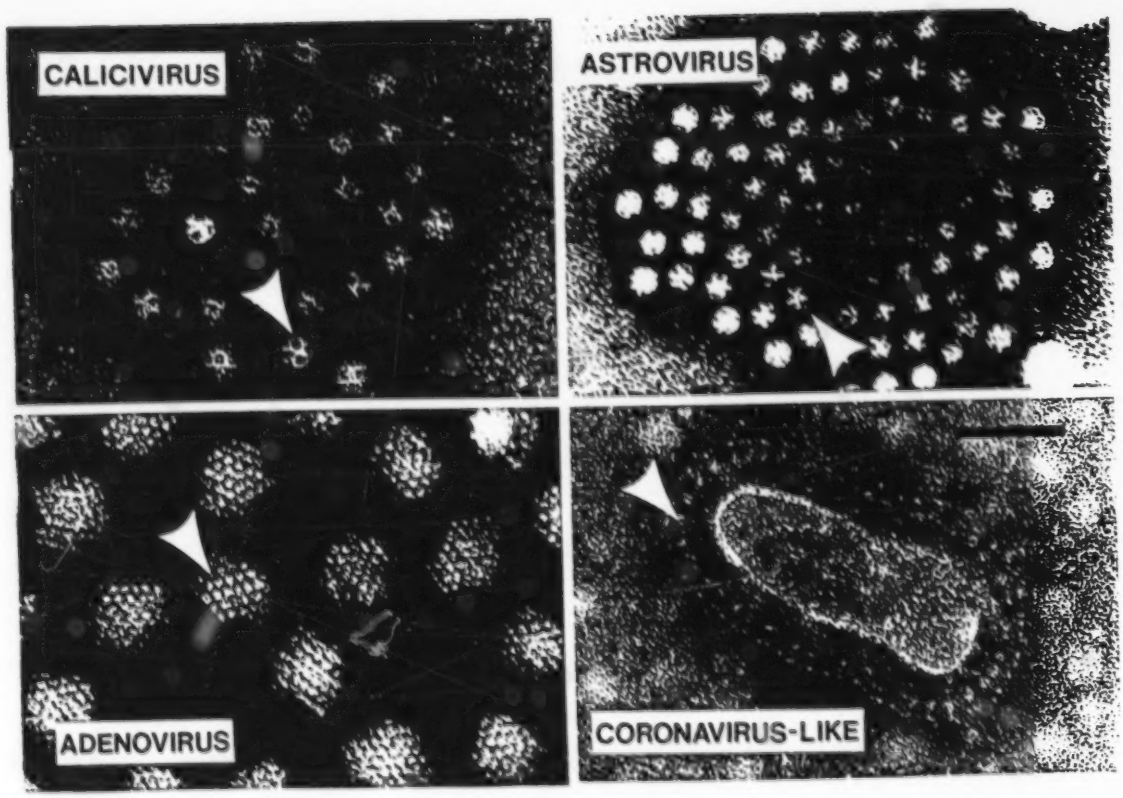
Location, Year	Ill	Remarks
Sweden, 1977	3,172	Small town
Brazil, 1980	812	Private school
Colorado, 1981	1,500	Two small communities
Soviet Union, 1981*	173	Settlement
E. Germany, 1981-2	11,600	Large town along river
China, 1982-3	13,311	Two outbreaks-atypical
Israel, 1984*	unknown	Community water system

*date of report

(adapted from Williams and Akin, 1986)

Table 4


NORWALK VIRUS
ROTAVIRUS


CALICIVIRUS
ASTROVIRUS
ADENOVIRUS
CORONAVIRUS-LIKE